A voltage-dependent K$^+$ current contributes to membrane potential of acutely isolated canine articular chondrocytes

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The electrophysiological properties of acutely isolated canine articular chondrocytes have been characterized using patch-clamp methods. The 'steady-state' current–voltage relationship (*I–V*) of single chondrocytes over the range of potentials from -100 to $+40$ mV was highly non-linear, showing strong outward rectification positive to the zero-current potential. Currents activated at membrane potentials negative to -50 mV were time independent, and the *I–V* from -100 to -60 mV was linear, corresponding to an apparent input resistance of 9.3 ± 1.4 GΩ ($n = 23$). The outwardly rectifying current was sensitive to the K$^+$ channel blocking ion tetraethylammonium (TEA), which had a 50% blocking concentration of 0.66 mM (at $+50$ mV). The 'TEA-sensitive' component of the outwardly rectifying current had time- and membrane potential-dependent properties, activated near -45 mV and was half-activated at -25 mV. The reversal potential of the 'TEA-sensitive' current with external K$^+$ concentration of 5 mM and internal concentration of 145 mM, was -84 mV, indicating that the current was primarily carried by K$^+$ ions. The resting membrane potential of isolated chondrocytes (-38.1 ± 1.4 mV; $n = 19$) was depolarized by 14.8 ± 0.9 mV by 25 mM TEA, which completely blocked the K$^+$ current of these cells. These data suggest that this voltage-sensitive K$^+$ channel has an important role in regulating the membrane potential of canine articular chondrocytes.

Articular chondrocytes play a central role in the production and maintenance of the cartilage in synovial joints. The metabolic state of the chondrocytes, which are solely responsible for the synthesis and degradation of the extracellular collagen and proteoglycan matrix of the cartilage, is strongly influenced by mechanical loading and the extracellular ionic and osmotic environment of the cell (Urban *et al.* 1993; Mobasheri *et al.* 2002). The mechanotransduction processes that link mechanical loading to the metabolic response of the chondrocytes are not completely understood, but factors such as mechanical distortion of the chondrocyte membrane and nucleus (Guilak, 2000), electric stimuli from piezoelectric effects and streaming potentials (Gu *et al.* 1998; Mow *et al.* 1999; Lai *et al.* 2000), effects produced by mechanical load-induced changes in matrix water content, ionic concentrations and pH (Mobasheri *et al.* 1998; Mow *et al.* 1999), and changes in chondrocyte membrane potential (Wright *et al.* 1996, 1997) have all been implicated.

A wide variety of ion channels and transporters are expressed in articular chondrocytes (Mobasheri *et al.* 1998, 2002). For example, electrophysiological studies have identified voltage-gated Na$^+$ channels (Sugimoto *et al.* 1996), voltage-gated and stretch-activated K$^+$ channels (Vittur *et al.* 1994; Sugimoto *et al.* 1996; Martina *et al.* 1997; Mozrzymas *et al.* 1997; Tsuga *et al.* 2002), and voltage-gated Cl$^-$ channels (Sugimoto *et al.* 1996; Tsuga *et al.* 2002) in articular chondrocytes. Indirect evidence, based primarily on the effects of pharmacological blockers, has also been obtained for the presence of both N- and L-type voltage-gated Ca^{2+} channels (Martina *et al.* 1997; Zuscik *et al.* 1997; Mow *et al.* 1999; Millward-Sadler *et al.* 2000) and stretch-activated cation channels (Wright *et al.* 1996; Yellowley *et al.* 1997, 2002; Guilak *et al.* 1999; Erickson *et al.* 2001) in articular and growth plate chondrocytes. Immunostaining studies have shown that mouse limb-bud chondrocytes express both epithelial Na$^+$ and voltage-gated Ca^{2+} channels (Shakibaei & Mobasheri, 2003). Articular chondrocytes also express a variety of

electrogenic and non-electrogenic Na^+, K^+ and Cl^- transporters, including Na^+-K^+-ATPase, Na^+-H^+ exchanger and $Na^+-K^+-2Cl^-$ cotransporter (Mobasheri *et al.* 1998; Trujillo *et al.* 1999). Despite this information, the ionic basis of the membrane potential in articular chondrocytes is not well understood at present.

The membrane potential of cell cultured rabbit articular chondrocytes was reported to be solely due to membrane Cl^- permeability, and changes in extracellular $[K^+]$ did not influence membrane potential (Tsuga *et al.* 2002). In contrast, the K^+ channel blocker tetraethylammonium (TEA) and high concentrations (150 mM) of extracellular K^+ depolarized human articular chondrocytes (Wohlrab & Hein, 2000; Wohlrab *et al.* 2002), implying that K^+ permeability made a contribution to the membrane potential in these cells. The membrane potential of chondrocytes in tissue culture has been shown to be sensitive to mechanical activity (Wright *et al.* 1992, 1996). Brief episodes of cyclic pressurization of human and sheep articular chondrocytes produced membrane hyperpolarization, an effect that was blocked by quinidine and apamin. The interpretation of these results was that pressurization induced activity of Ca^{2+}-dependent K^+ channels, which produced membrane hyperpolarization (Wright *et al.* 1992, 1996).

In this study, the membrane currents in acutely isolated canine articular chondrocytes have been studied using patch clamp methods. The use of acutely isolated cells reduces the influences of cell dedifferentiation that may occur in cell culture conditions. The current–voltage relation of these acutely isolated cells showed strong outward rectification, which was produced by a time- and voltage-dependent K^+ current that was sensitive to relatively low concentrations of TEA ($K_d = 0.66$ mM). The biophysical properties of this current were characterized and these data suggested that this K^+ current could contribute significantly to the membrane potential of acutely isolated canine articular chondrocytes. This was confirmed by showing that concentrations of TEA which were sufficient to block the K^+ current resulted in significant membrane depolarization.

Methods

Isolation of chondrocytes

Articular cartilage was removed from the stifle joint of healthy, skeletally mature mixed-breed canines of either sex, aged 2–3 years (body mass 15–25 kg). Animals were cared for under the supervision of a veterinarian according to the guidelines of The Canadian Council on Animal Care

with the approval of the University of Calgary institutional animal care committee. Animals were killed by intravenous bolus injection of barbiturate (Euthanyl). Chips of articular cartilage were removed and incubated in cell culture medium (DMEM F12 with 10% FBS at 37°C; Invitrogen Corp., Carlsbad, CA, USA) for 12–24 h to ensure sterile conditions. Populations of isolated cells were prepared by digesting chips in a series of solutions containing 0.25% hyaluronidase (room temperature, for 5 min), 0.8% pronase (1 h at 37°C, with stirring) and 0.4% collagenase (20 min at 37°C, with stirring). Aliquots of cell suspension were plated onto glass-bottomed electrophysiological recording chambers for 30–60 min, which allowed the isolated chondrocytes to lightly adhere to the glass before they were superfused with bath solutions. Cell preparations were used for no more than 4 h after isolation.

Electrophysiology

Whole-cell patch-clamp methods (Hamill *et al.* 1981) were used to measure membrane currents from single voltage-clamped chondrocytes, and to measure membrane potentials in current clamp. Patch pipettes were pulled from 1.5 mm o.d./1.2 mm i.d. borosilicate glass (Sutter Instrument Co., Novato, CA, USA) and lightly fire polished. The resistance of the pipettes when filled with pipette solution was in the range 3–8 MΩ. Membrane currents and potentials were recorded with a MultiClamp 700A amplifier (Axon Instruments, Union City, CA, USA). Currents and voltages were digitized, and voltage-clamp protocols were generated with a Digidata 1322A data-acquisition interface controlled with pCLAMP8 software (Axon Instruments).

The capacitance of acutely isolated canine articular chondrocytes ranged from 1.8 to 11 pF, and averaged 5.78 ± 0.11 pF for a sample of 203 cells from 17 different cell preparations. The pipette-to-cell seal resistance, measured before rupturing the membrane patch in the pipette, averaged 28.2 ± 2.5 GΩ ($n = 58$).

Cells were superfused with a modified Tyrode solution (see below) at 1–2 ml min^{-1}. The temperature of the superfusion solution was 23 ± 2°C. Solutions containing drugs were delivered to cells using a multibarrelled local superfusion device, which used solenoid valves to control solution flow (ALA Scientific Instruments Inc., Westbury, NY, USA).

Use of unequal $[Cl^-]$ in the patch pipette and bath solution (see below) produced a liquid junction potential which resulted in an approximately +10 mV offset of the recorded membrane potentials (Neher, 1992). Note that all

membrane potentials in the data presented in Results were 'corrected' for the liquid junction potential by subtracting 10 mV from the values recorded during the experiments.

Solutions and drugs

Modified Tyrode solution consisted of (mM): NaCl, 140; KCl, 5; CaCl$_2$, 1.8; MgCl$_2$, 1.0; Hepes, 5; glucose, 10. The pH was adjusted to 7.4 with 1 M NaOH. In solutions containing tetraethylammonium chloride (TEACl), NaCl was replaced by an equal concentration of TEACl. The patch pipette solution consisted of (mM): potassium aspartate, 110; KCl, 20; MgCl$_2$, 4; K$_2$ATP, 4; Na$_2$GTP, 0.1; sodium phosphocreatine, 6.6; EGTA, 5; Hepes, 5. The pH was adjusted to 7.2 with 1 M KOH.

Statistics

Mean values are shown with s.e.m. Sets of data were least-squares-fitted to Hill and Boltzmann equations using 'Sigmaplot' (SPSS Inc. Chicago, IL, USA).

Results

Voltage-dependent membrane currents in canine articular chondrocytes

Figure 1*A* illustrates a representative example of a family of membrane currents from a voltage-clamped articular chondrocyte. The membrane potential was stepped for 1 s from a holding potential of −55 mV to a series of potentials between +40 and −100 mV. Steps to potentials between −50 and −100 mV produced small 'instantaneous', time-independent jumps in current. For steps positive to −40 mV, however, outward currents had a time-dependent component which increased in amplitude as the membrane depolarization increased. The rate of activation of the time-dependent current increased with increased membrane depolarization, and over the range of membrane potentials from −30 to +40 mV, the one-half activation time of the time-dependent component of the currents shown in Fig. 1*A* increased from about 27 ms to 2.2 ms, respectively. After the end of the depolarizing step, the currents declined (deactivated) back to holding current levels within about 50 ms. Figure 1*B* shows a plot of
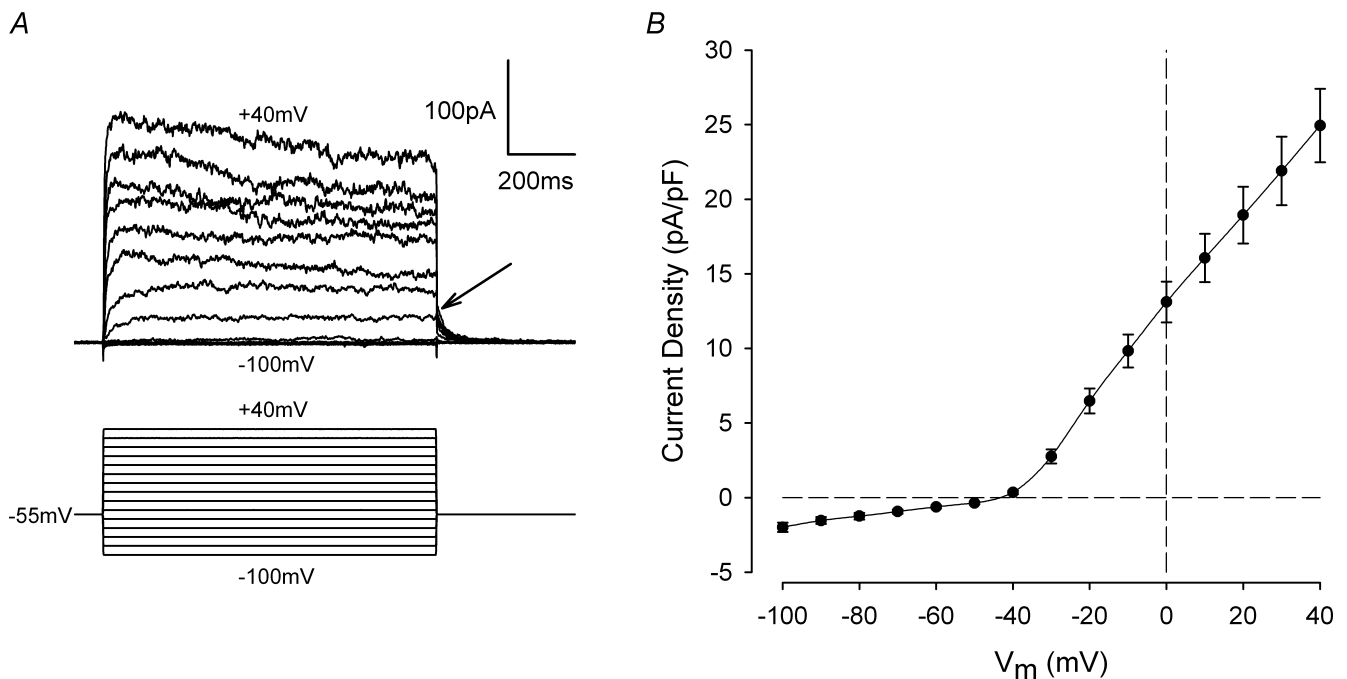

Figure 1. Current–voltage relation of acutely isolated chondrocytes
A, representative family of membrane currents from a voltage-clamped articular chondrocyte. The voltage-clamp protocol is shown below the current records. Holding potential (V_h) was −55 mV, and the membrane potential was stepped from +40 to −100 mV in 10 mV increments at 3 s intervals. Arrow indicates deactivation of outward currents at the end of the depolarizing steps. Cell capacitance was 6.5 pF. *B*, mean peak current–voltage relationship from 24 cells (5 different cell preparations). Currents from each cell were normalized to cell capacitance, and then averaged. The mean cell capacitance of the 24 cells was 5.8 ± 0.3 pF.

the mean peak outward current density *versus* membrane potential (*I–V* relation) for 24 different cells. Currents from each cell were normalized to cell capacitance, and the current density values were averaged. The *I–V* plot showed very pronounced outward rectification beginning near −45 mV, and the outward currents monotonically increased as membrane potential became more positive. For membrane potentials negative to about −50 mV, the *I–V* relation was linear, and the mean apparent input resistance of the cells, from the slope of the *I–V* between −60 and −100 mV, was 9.3 ± 1.4 GΩ (*n* = 23).

Outward currents in articular chondrocytes are sensitive to TEA

The K⁺ channel-blocking ion TEA⁺ strongly inhibited outward membrane currents in articular chondrocytes.

Figure 2*A* illustrates the effect of a wide range of TEA concentrations (0.25–25 mM) on the outward currents produced by a voltage-clamp step to +30 mV. In this chondrocyte, 0.25, 2.5 and 25 mM TEA reduced total outward current amplitude by about 33, 73 and 88%, respectively. The current in the presence of 0.25 and 2.5 mM TEA exhibited time-dependent activation with a time course that was similar to that of the control current, but in the presence of 25 mM TEA, the current appeared to activate 'instantaneously' and was constant in magnitude throughout the voltage-clamp step. These results demonstrate that 25 mM TEA completely blocked the voltage-dependent outward current, leaving a time-independent background current. Figure 2*A* also shows that the 'TEA-sensitive' component of the outward current, obtained by subtraction of the current in 25 mM
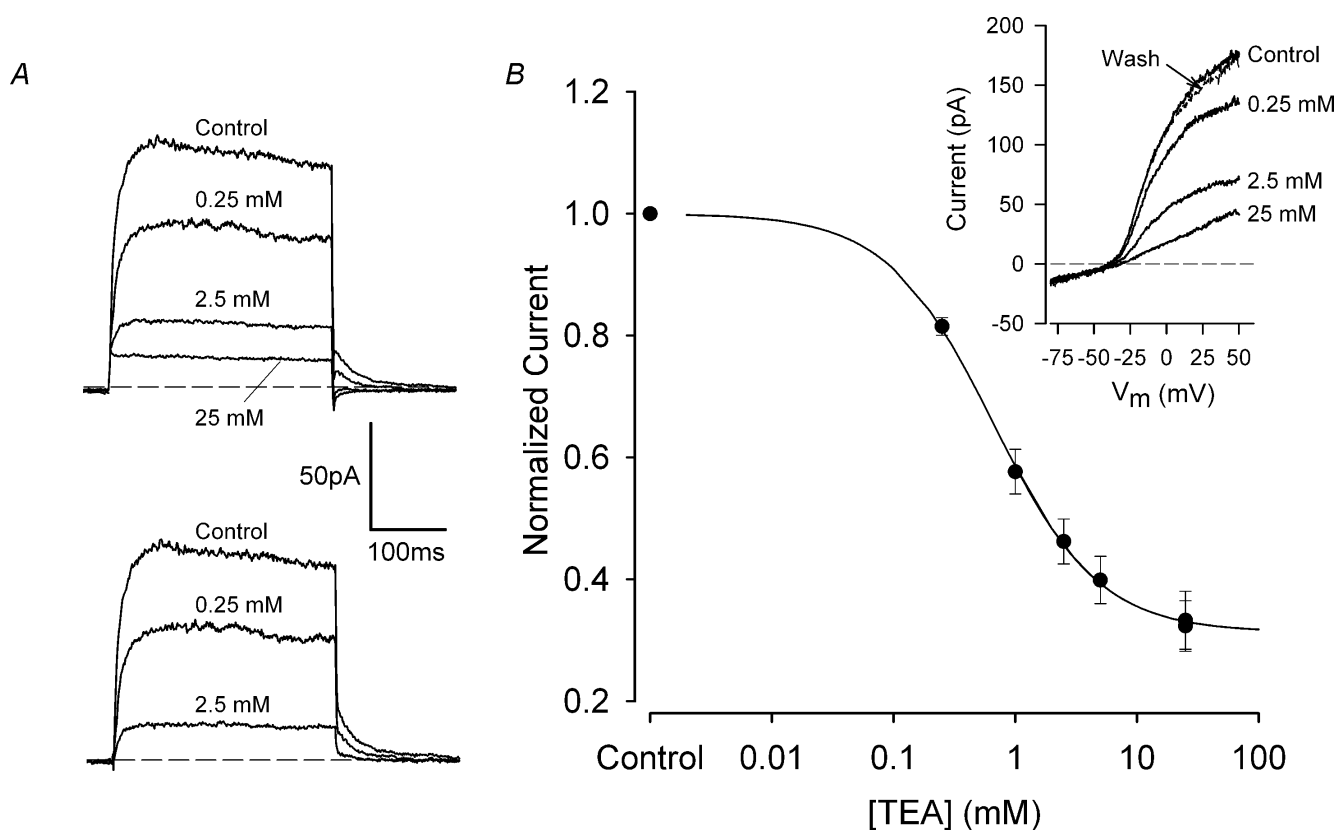

Figure 2. Dose–response for the effect of tetraethylammonium (TEA) on outward currents
A, effect of tetraethylammonium chloride (TEA) on outward currents of a voltage-clamped chondrocyte. Voltage-clamp step was +30 mV, V_h = −55 mV. Currents were recorded in control solution, and solution containing 0.25, 2.5 and 25 mM TEA. Four to six records were averaged at each TEA concentration. The lower set of records shows the 'TEA-sensitive' current, obtained by subtraction of the current in 25 mM TEA. *B*, TEA dose–response relationship. Inset: current–voltage relationships were generated with a 1 s voltage 'ramp' from −100 mV to +50 mV (V_h −55 mV), in control and TEA-containing solutions. Plot: mean, normalized current *versus* TEA concentration. The total current at +50 mV for each TEA concentration was normalized to control for each cell, and normalized values were averaged. Two batches of cells were used; the number of cells at each concentration of TEA was in the range 7–20. Continuous line is best fit of the Hill equation, viz., $I = A/(1 + [TEA]/K_d) + (1 − A)$, where *A* is the fraction of current sensitive to TEA and K_d is the 50% blocking dose of TEA. The best-fit line had A = 0.687 ± 0.004 and K_d = 0.66 ± 0.02 mM.

TEA, had a similar time course to the control current, suggesting that the blocking action of TEA was not primarily on open channels. Figure 2*B* shows the dose–response relation for the blocking action of TEA on the outward current. These data were obtained from *I–V* relations generated by voltage ramps, as shown in the inset to Fig. 2*B*. The plot in Fig. 2*B* is the relationship between normalized total current at +50 mV and concentration of TEA. The current at +50 mV, in each concentration of TEA, was normalized for each cell to its control value, and these normalized values were then averaged. The data in Fig. 2*B* were pooled from two different batches of cells; in one batch the TEA concentrations used were 1, 10 and 25 mM, and in the other the concentrations were 0.25, 2.5 and 25 mM. The continuous line in Fig. 2*B* shows the best-fit Hill equation (see figure legend) with $n = 1$. The 50% blocking dose of TEA from this equation was 0.66 ± 0.02 mM, and 68.7% of the total outward current at +50 mV was TEA sensitive.

Figure 3*A* shows the effect of 25 mM TEA on a family of currents produced by voltage steps from +40 to −100 mV. Outward currents were strongly inhibited by TEA, while currents negative to the holding potential, −55 mV, were not significantly affected. Figure 3*B* compares the *I–V* relationships for membrane currents that were sensitive and insensitive to TEA. The 'TEA-insensitive' current (the
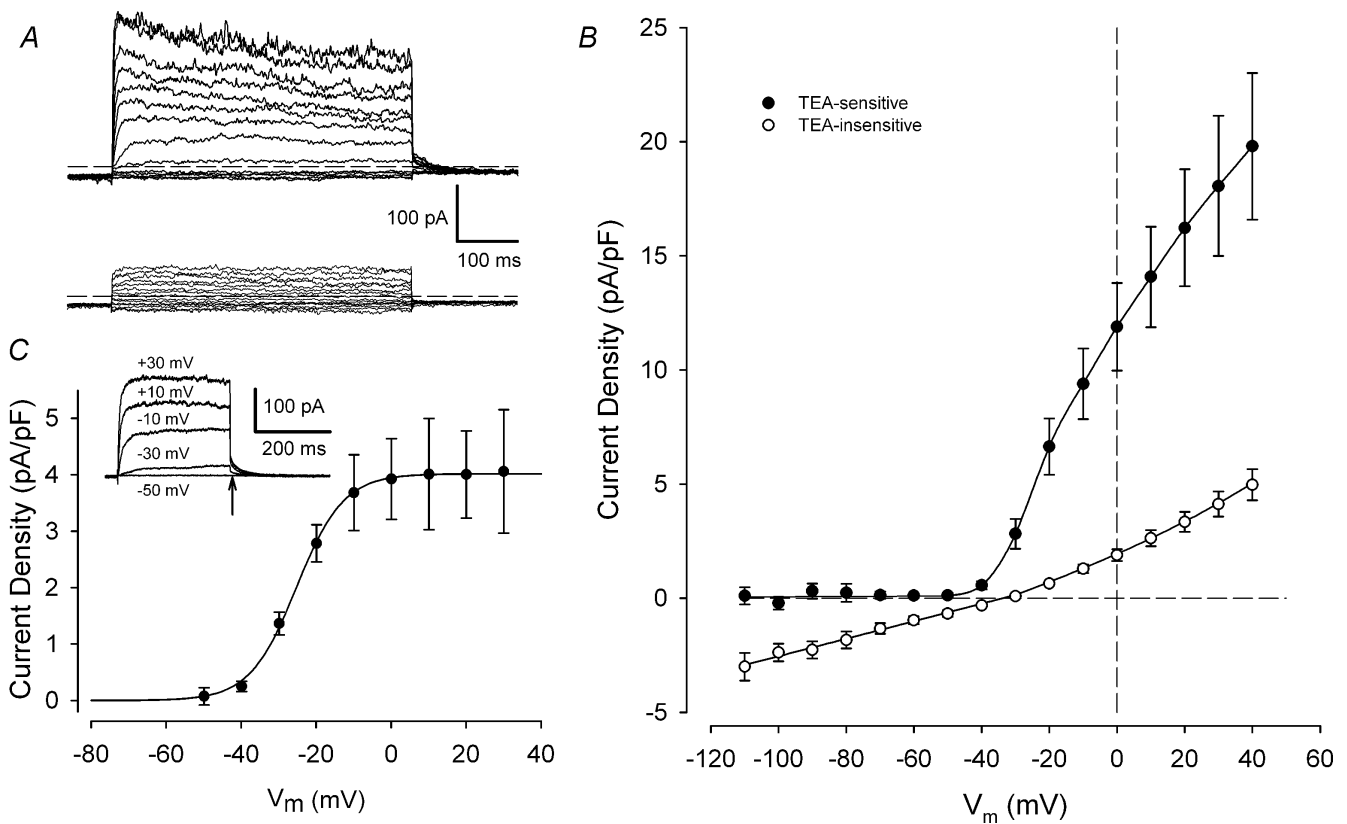

Figure 3. Current–voltage relations for 'TEA-sensitive' and 'TEA-insensitive' membrane currents
A, family of currents from voltage-clamped chondrocyte before (upper records) and after (bottom records) exposure to 25 mM TEA. Voltage-clamp protocol was similar to that shown in Fig. 1*A*, except the depolarizing steps were 500 ms in duration. *B*, mean *I–V* relationships for 'TEA-sensitive' and 'TEA-insensitive' currents in 15 chondrocytes. 'TEA-insensitive' currents were the currents remaining in the presence of 25 mM TEA; 'TEA-sensitive' currents were obtained by subtraction of control currents from 'TEA-insensitive' currents. Currents from each cell were normalized to cell capacitance, and then averaged. Mean cell capacitance was 5.3 ± 0.4 pF ($n = 15$). *C*, voltage dependence of activation of 'TEA-sensitive' current. The amplitude of the deactivating tail current (at a membrane potential of −55 mV) was measured as a function of the depolarizing step. Inset shows an example of a family 'TEA-sensitive' currents for a series of 300 ms depolarizing steps from −50 to +30 mV. Tail current amplitude was measured at the end of the depolarizing step (arrow). The plot shows mean ($\pm$ S.E.M.) tail current density (pA pF^{-1}) as a function of membrane potential for 8 cells. The continuous line is the best-fit Boltzmann function (see text), with $V_{1/2} = -25.2 \pm 0.3$ mV and $S_{1/2} = 6.3 \pm 0.3$ mV.

current remaining in 25 mM TEA) showed weak outward rectification, with an apparent reversal potential between -30 and -35 mV. Membrane currents at potentials between -50 and -110 mV were unaffected by TEA. The 'TEA-sensitive' current (obtained by subtraction of control currents from the 'TEA-insensitive' currents) was undetectable at membrane potentials negative to -50 mV, but began to activate near -45 mV. This current increased steeply with membrane depolarization until about -10 mV, and it continued to increase for more positive depolarizations.

The voltage dependence of activation of the 'TEA-sensitive' current is shown in Fig. 3*C*. Activation was measured from the amplitude of the deactivating tail current, as a function of the depolarizing step (see inset to Fig. 3*C*). The amplitude of the tail current was nearly zero at -50 mV, but it increased rapidly with membrane depolarization over the range -40 mV to about -10 mV, and then remained constant for potentials more positive than -10 mV. The mean tail current amplitude *versus* potential was fitted to a Boltzmann function, viz.

$$I = I_{max}/[1 + \exp(-(V_m - V_{1/2})/S_{1/2})]$$

where I_{max} is the maximal tail current amplitude, V_m is membrane potential, $V_{1/2}$ is the potential for one-half maximal tail current and S_h is a slope factor at $V_{1/2}$. The best fit of this equation to the mean tail current data in Fig. 3*C* had $I_{max} = 4.0 \pm 0.02$ pA pF^{-1}, $V_{1/2} = -25.2 \pm 0.3$ mV and $S_{1/2} = 6.3 \pm 0.3$ mV.

Figure 4*A* compares the effects on chondrocyte membrane currents of another K$^+$ channel blocker, 4-aminopyridine (4-AP), with TEA. A 'ramp' voltage-clamp protocol (cf. Fig. 2*B*) was used to generate an *I–V* relation. 4-AP (1 mM) reduced the magnitude of the TEA-sensitive current by about 31% at $+50$ mV. Similar results were obtained in three other cells; the mean reduction in TEA-sensitive current at $+50$ mV by 1 mM 4-AP was $26.5 \pm 6.3\%$ ($n = 4$).

Figure 4*B* shows that the *I–V* relation was only slightly altered by complete removal of extracellular Ca^{2+} (replacement with equimolar Mg^{2+}). TEA-sensitive current magnitude was not significantly changed, but the current appeared to activate at slightly more negative membrane potentials. Similar results were obtained in two other cells. The patch-pipette solution contained 5 mM EGTA, without any added Ca^{2+} (Methods); thus internal free [Ca^{2+}] was very low (<10 nM). Consequently, entry of Ca^{2+} into the cell cytoplasm via, for example, voltage-gated Ca^{2+} channels, would be unlikely to result in an increase in intracellular [Ca^{2+}]. In summary, the data in Fig. 4*B*

indicate that it is improbable that activation of the TEA-sensitive current is dependent on intracellular [Ca^{2+}].

TEA-sensitive current is a K$^+$ current

The data in Fig. 4*C* show that the TEA-sensitive, voltage-gated current is carried by K$^+$. Tail currents which followed a depolarizing step were recorded at membrane potentials between -65 and -95 mV. Tail current polarity reversed at a potential of -84.3 mV. The Nernst potential for K$^+$ in the extra- and intracellular solutions was -84 mV. The only other major ion in the solutions was Cl$^-$, whose Nernst potential was -42 mV. The sensitivity of the voltage-gated current to TEA, and its reversal potential at the K$^+$ Nernst potential clearly identifies it as a K$^+$ current.

TEA produces membrane depolarization of chondrocytes

Figure 5*A* shows the effect of 25 mM TEA on the resting membrane potential of an isolated chondrocyte, recorded in current clamp conditions. The cell was exposed briefly (5–10 s) to solution containing 25 mM TEA during three successive intervals. In each case, exposure of the cell to TEA produced a very rapid depolarization of the membrane from a resting potential of approximately -42 mV to about -30 mV. The depolarization was readily reversible on removal of TEA. Prior to recording the membrane potentials, *I–V* relations were recorded in the same cell in the absence and presence of 25 mM TEA (Fig. 5*B*). As shown by data presented above (e.g. Fig. 2) the voltage-dependent outward currents were completely suppressed by TEA. The resting membrane potential of 19 different chondrocytes (from 3 different cell preparations) in control solution was -38.1 ± 1.4 mV. TEA (25 mM) produced an average depolarization of 14.8 ± 0.9 mV in the same 19 chondrocytes.

Discussion

Properties of voltage-gated K$^+$ current in acutely isolated canine articular chondrocytes

The biophysical properties of a time- and voltage-dependent K$^+$ current in acutely isolated canine articular chondrocytes have been characterized in this study. This K$^+$ current was activated over membrane potentials from about -45 mV to approximately -10 mV. This current exhibited many of the characteristics of a 'delayed rectifier' K$^+$ current, with rapid activation and very slow inactivation. Reversal of the current occurred close to

the predicted Nernst potential for K$^+$, demonstrating a high selectivity of the channel for K$^+$. Pharmacological studies showed that this current was sensitive to TEA, with a half-blocking concentration of 0.66 mM, and that 4-aminopyridine (1 mM) blocked about 27% of the current. Removal of extracellular Ca^{2+} had little effect on the current. Since intracellular [Ca^{2+}] was strongly buffered by EGTA in the patch-clamp pipette, this current was probably not activated by changes in intracellular [Ca^{2+}]. Isolated chondrocytes were depolarized by TEA

(25 mM), indicating that this K$^+$ current can contribute to the membrane potential of canine chondrocytes.

K$^+$ channels and currents in chondrocytes

Patch-clamp studies have identified a variety of K$^+$ channels and currents in cell cultured articular and growth plate chondrocytes. Large conductance, Ca^{2+}-activated K$^+$ channels were identified in porcine (Grandolfo *et al.* 1990, 1992) and chicken (Long & Walsh, 1994)
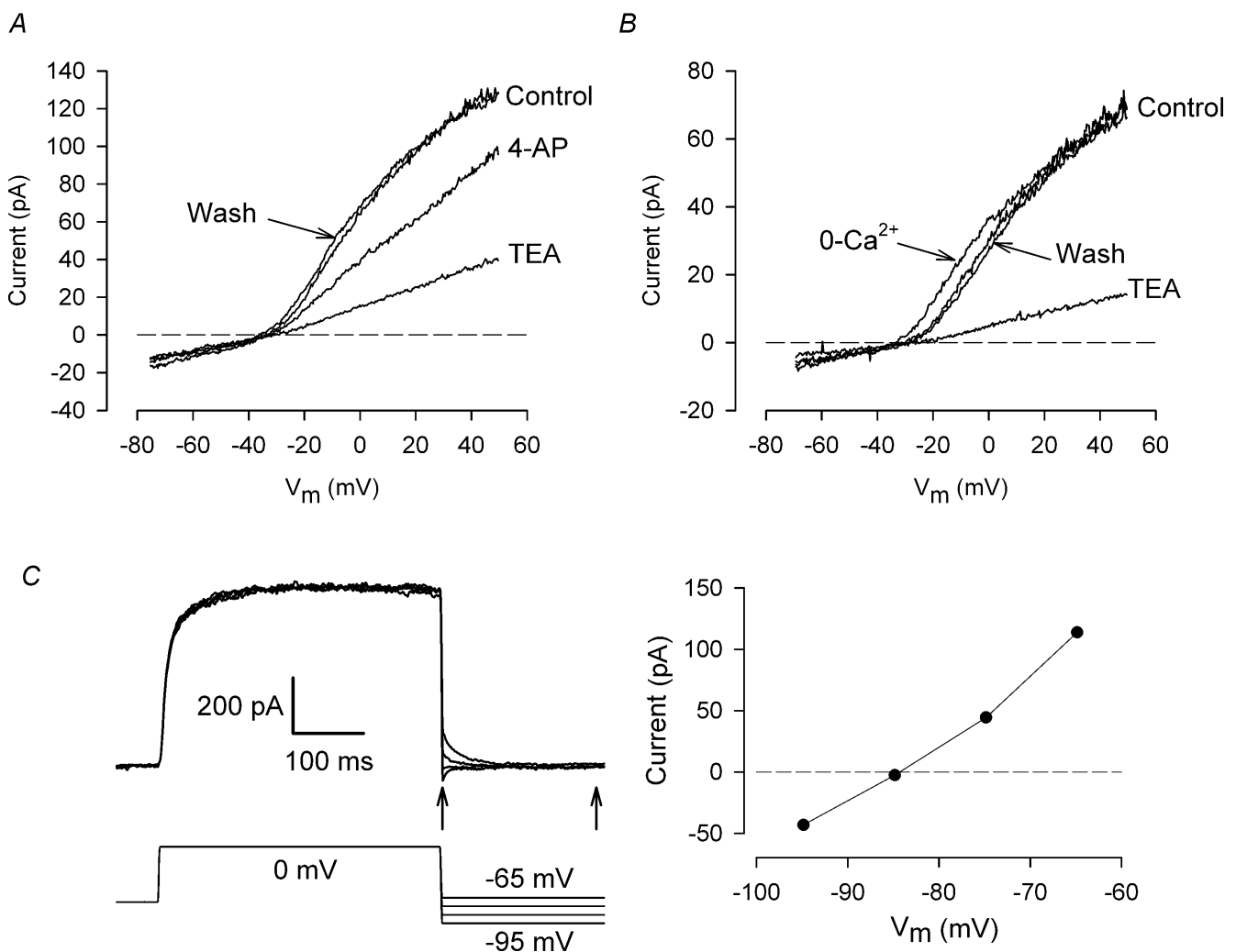

Figure 4. Pharmacology and reversal potential of isolated chondrocytes
A, effect of 4-aminopyridine (4-AP) on currents. *I–V* relationships were produced with a ramp voltage clamp (cf. Fig. 2). Following the control *I–V*, the cell was exposed to 25 mM TEA. An *I–V* was recorded after removing TEA (data not shown), then the cell was exposed to 1 mM 4-AP. 'Wash' shows the *I–V* after washout of 4-AP. *B*, effect of removing extracellular Ca^{2+}. Ca^{2+} in control solution (see Methods) was replaced by equimolar Mg^{2+}. Control *I–V* was followed by 0 Ca^{2+}, wash (data not shown), 25 mM TEA, and second wash. *C*, reversal potential of current. Tail currents following depolarizing step to 0 mV were recorded at potentials between −95 and −65 mV. The magnitude of the tail current was taken as the difference in current immediately after the end of the depolarizing step, and after the currents had reached a 'steady state' (arrows). Plot at right shows tail current magnitude *versus* membrane potential.

growth plate chondrocytes. Two types of voltage-gated K$^+$ channels with different single-channel conductances were recorded from porcine and equine articular chondrocytes (Mozrzymas *et al.* 1994, 1997; Vittur *et al.* 1994). A large conductance, stretch-activated K$^+$ channel was recorded from porcine articular chondrocytes (Martina *et al.* 1997). Whole-cell current recordings from porcine (Mozrzymas *et al.* 1997) and rabbit (Sugimoto *et al.* 1996) articular chondrocytes identified K$^+$ currents with delayed rectifier-like properties. The K$^+$ currents in rabbit chondrocytes were partially blocked by 4-AP (5 mM, 92% block) and TEA (5 mM, 58% block). A similar delayed rectifier-like K$^+$ current was recorded from cell cultured chicken growth plate chondrocytes (Walsh *et al.* 1992); this current was strongly inhibited by 4-AP (82%; 1 mM) and charybdotoxin ($\sim$90%; 10 nM), but was not significantly affected by TEA (10 mM). None of these K$^+$ currents or channels appears to have properties that are identical to the voltage-gated K$^+$ currents recorded from acutely isolated canine articular chondrocytes in this study.

The K$^+$ current recorded from canine articular chondrocytes appears to most closely resemble the K$^+$ currents recorded from an *in situ* preparation of porcine growth plate chondrocytes (Lee *et al.* 2001). Chondrocytes embedded in pieces of growth plate were exposed by enzymatic digestion of the surface of the cartilage, and then whole-cell patch clamped. A delayed rectifier-like K$^+$ current was the principal voltage-dependent current recorded from cells in this preparation. The current was half-activated at about -11 mV, and was completely blocked by 20 mM TEA. The current density depended on whether the recordings were made from resting, proliferative or hypertrophic chondrocytes. At a membrane potential of 0 mV, current density varied from about 0.3 pA μm^{-2} for resting and hypertrophic cells to 0.6 pA μm^{-2} for proliferative cells. Assuming a specific membrane capacitance of 1 μF cm^{-2} (Hille, 2001), this corresponds to a current density of about 30–60 pA pF^{-1}. This compares favourably with a current density of $\sim$12 pA pF^{-1} for the TEA-sensitive current of canine chondrocytes at 0 mV (Fig. 3*B*).
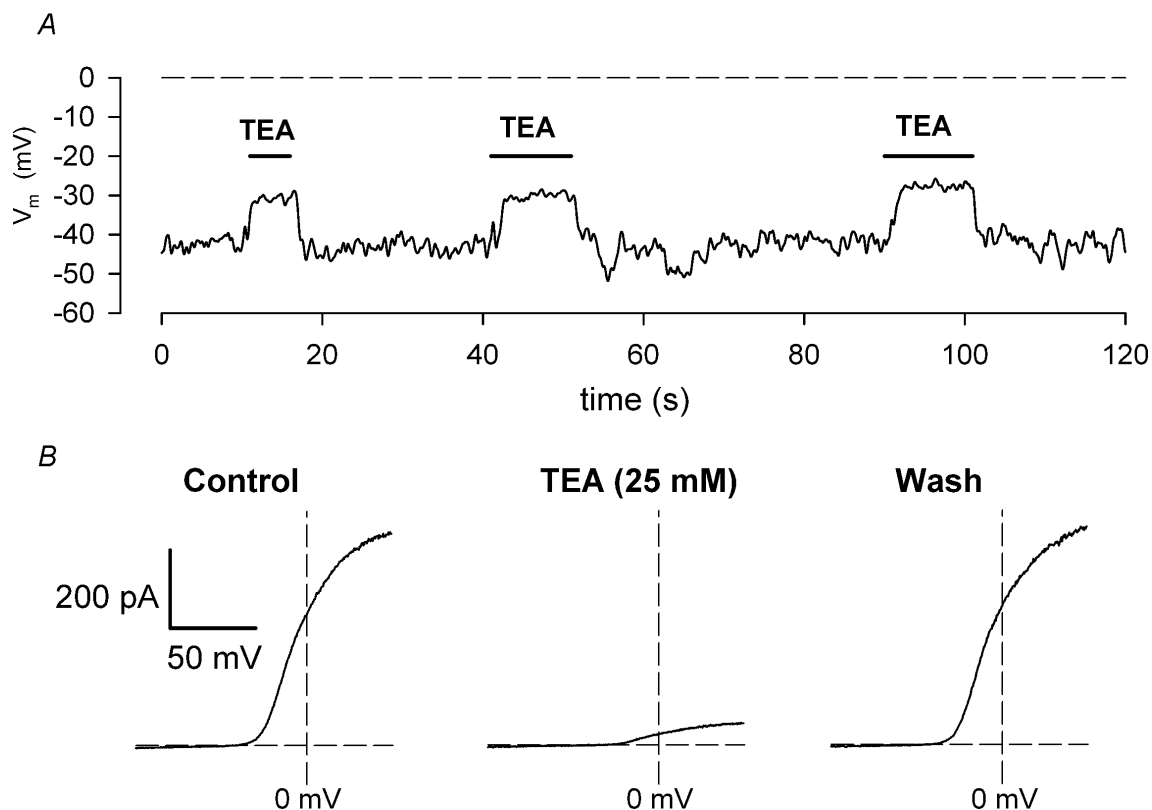

Figure 5.
A, effect of TEA on resting membrane potential of an isolated chondrocyte. The plot is a continuous recording of the membrane potential. Solution containing 25 mM TEA was applied to the cell for 3 successive intervals, as shown. *B*, current–voltage relations from the same cell, in control (left), in 25 mM TEA (centre) and after washout of TEA (right). The *I–V* relations were generated using a voltage ramp protocol (cf. Fig. 2).

Chondrocyte membrane potential – a role for K$^+$ channels?

The ionic basis of chondrocyte membrane potential is not well understood, and has been investigated in detail only for cell cultured rabbit articular chondrocytes (Tsuga *et al.* 2002). Membrane potential, measured using patch-clamp methods, was determined by the Nernst potential for intra- and extracellular [Cl$^-$]. Single-channel recordings identified large conductance ($>$200 pS), voltage-gated Cl$^-$ channels which were probably the basis for the membrane Cl$^-$ permeability (Tsuga *et al.* 2002). Large changes in extracellular [K$^+$] (5–40 mM) had no significant effect on membrane potential, indicating that K$^+$ ions did not contribute to the membrane potential. This is in contrast to cell cultured human articular chondrocytes in which membrane potential, measured with potential-sensitive optical dyes (Wohlrab & Hein, 2000; Wohlrab *et al.* 2002), was significantly depolarized by increased extracellular [K$^+$] and high concentrations of TEA (20–40 mM), indicating the involvement of K$^+$ channels in determining membrane potential. Depolarization of acutely isolated canine chondrocytes by 25 mM TEA (Fig. 5), which completely blocked the voltage-gated K$^+$ current in these cells (Figs 2 and 5), provides strong evidence that K$^+$ channels contribute significantly to membrane potential under the conditions of these experiments.

The membrane potential of rabbit growth plate chondrocytes, measured *in situ* with intracellular micro-electrodes in pieces of cartilage (Edelman *et al.* 1985), was dependent on the stage of differentiation of the chondrocyte, approximately −36 mV for hypertrophic cells and −56 mV for proliferative cells. It is interesting that the density of the delayed rectifier K$^+$ current recorded from *in situ* porcine growth plate chondrocytes was significantly greater for proliferative cells than for hypertrophic cells (Lee *et al.* 2001); this suggests that the difference in membrane potential between proliferative and hypertrophic growth plate chondrocytes may result from the difference in current density of the voltage-gated K$^+$ current in these cells. Although the half-activation potential of the current (approximately −11 mV) was much more positive than the membrane potential (i.e. −36 to −56 mV), activation of the current began between −60 and −50 mV (Lee *et al.* 2001). Because of the very high membrane resistance of chondrocytes, 'steady-state' activation of only a very small amount of K$^+$ current would significantly hyperpolarize these cells. It is probable that the TEA-sensitive, voltage-gated K$^+$ current contributes to resting membrane potential of isolated canine chondrocytes by this mechanism. Figure 3*C* shows

that the TEA-sensitive and TEA-insensitive currents were equal and opposite in magnitude near −40 mV, which was about 10 mV more negative than the zero-current potential of the TEA-insensitive current. Hence, block of the TEA-sensitive K$^+$ current would be expected to produce a membrane depolarization of approximately 10 mV, which is in good agreement with the mean TEA-induced depolarization of 14.8 mV (cf. Fig. 5). The data in Fig. 3*C* shows that only about 12% of the maximal K$^+$ conductance was activated at the mean resting membrane potential, −38 mV.

Limitations of this study

Dissociated chondrocytes readily dedifferentiate in mono-layer cell culture conditions, and the expression of cell matrix proteins such as collagen and proteoglycans changes in culture (Mayne *et al.* 1976; Pacifici *et al.* 1981; Benya & Shaffer, 1982; Plaas *et al.* 1983). There are indications that expression and properties of ion channels may also change in cell cultured chondrocytes. For example, the conductance of K$^+$ channels recorded from cultured porcine growth plate chondrocytes increased with time in culture, as the cultures changed from isolated cells to confluence (Grandolfo *et al.* 1990), and in mono-layer cell cultured porcine articular chondrocytes, stretch-activated K$^+$ channels were recorded most frequently in cells that had been in culture for 4–6 days (Martina *et al.* 1997). In this study, we have attempted to minimize such sources of variability by using chondrocytes which were acutely isolated from intact cartilage for at most a few hours before use. However, mechanical activity also profoundly affects chondrocyte metabolism, changing for example, proteoglycan, DNA and cAMP synthesis (e.g. Bourret & Rodan, 1976; Veldhuijzen *et al.* 1979; Sah *et al.* 1989; Buschmann *et al.* 1995; Lee & Bader, 1997). Consequently, storage of chips of cartilage in culture conditions for 12–24 h (see Methods) without appropriate mechanical stimulation before isolation of the chondrocytes may potentially produce changes in ion channel expression.

The *in situ* extracellular osmotic and ionic environment of articular chondrocytes is very different from that of most cells. The presence of immobile negative charges on the cartilage matrix proteoglycans results in cation concentrations in cartilage that are elevated compared to those in serum/synovial fluid, whereas anion concentrations are reduced. The concentrations of Na$^+$ (250–350 mM), K$^+$ (8–10 mM) and Ca^{2+} (15–20 mM) in articular cartilage are 2–10 times greater than their respective concentrations in serum/synovial fluid, whereas Cl$^-$ concentration in cartilage (60–90 mM) is roughly

1.5–2 times less (Maroudas, 1979). Moreover, the pH of cartilage is 6.9–7.1 (Gray *et al.* 1988), significantly lower than the pH of 7.4 of serum. The elevated cation concentration results in an increased osmotic pressure of the cartilage fluid, which measures 380–450 mosmol $(\text{kg } H_2O)^{-1}$: this compares with an osmolality of about 280 mosmol $(\text{kg } H_2O)^{-1}$ for the Hepes-buffered solution used in the patch-clamp experiments. It is unclear how the properties of the voltage-gated K^+ channel recorded from isolated chondrocytes might be modified in the presence of elevated cation and reduced anion concentrations, and osmotic pressures and pH that are more representative of intact cartilage.

Summary

This study demonstrates that a voltage-gated, Ca^{2+}-independent K^+ current with a substantial current density can be recorded from acutely isolated canine articular chondrocytes, and this K^+ current makes an important contribution to chondrocyte membrane potential. Further experiments will be necessary to determine the properties and role of this current in regulating chondrocyte membrane potential in ionic and osmotic conditions that more closely mimic those found *in situ* in cartilage.

The majority of electrophysiological studies of chondrocytes to date have used cells in monolayer culture. It would be of interest in future studies to measure the electrophysiological properties of chondrocytes that have been maintained in three-dimensional culture conditions such as agarose (Benya & Shaffer, 1982) or alginate (Bonaventure *et al.* 1994) gels that are known to preserve chondrocyte phenotype better than monolayer cultures.

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